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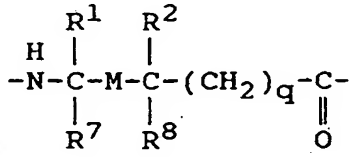
(54) **Factor IIa inhibitors.**

(57) Disclosed are compounds of the formula:

X Y Z A P Q T

which are highly potent, selective factor IIa inhibitors. X, when present, may be connected to T, may be H, CH_3, an acyl group, or a general protective group. Y is D-DPA, D-Phe, D-Val, D-Ile, D-Nle, a phenylsulfonyl, Dansyl, or 8-(1,2,3,4-tetrahydroquinolinosulfonyl) compound. Z, when present, is Gly, L/D-Pro, L/D-Ala, L/D-Leu, aminoisobutyric acid, a substituted or unsubstituted L/D-Pro ring homologue, or L/D-Val.

A may be



wherein M is $-CO-CF_2-(CO)_s-$, $-(CO)_d-NH-$, $-(CO)_d-(CH_2)_p-$, or $-CH(OH)-(CH_2)_p-$, d is 0 = 2, p and q are 0-5, and s is 0 or 1, or A is a proline-like group. P and Q (if Q is present) are substituted or unsubstituted amino acids selected from the group consisting of L/D-Phe, L/D-Cha, L/D-Nal(1), L/D-Nal(2), L/D-phenylglycinyl, L/D-Leu, L/D-Ile, L/D-Nle, L/D-Arg, L/D-Lys, or L/D-His. T may be connected to X, or may be -OH, $-OR^4$, $-NH_2$, $-NHR^4$, or $-NR^4R^5$, wherein R^4 and R^5 are independently selected from alkyl, aryl, (ar)alkyl, and wherein R^4 and R^5 can be cyclically bonded one to another.

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Technical Field This invention relates to certain medicinal compounds and their use as anti-factor IIa inhibitors.

Background Art: Attempts have been made in the past to make efficacious anticoagulants. One type of anticoagulant acts to prevent the production of fibrin by somehow preventing the production of an enzyme called thrombin ("factor IIa"). This is done since thrombin acts to catalyze the cleavage of fibrinogen to form fibrin, the material from which blood clots are formed.

For example, U.S. Patent No. 4,857,508 describes certain "RGD" peptide derivatives which assertedly inhibit platelet aggregation. These peptide derivatives are believed to act by antagonizing interactions between fibrinogen and/or extracellular matrix proteins and the platelet gpIIb/IIIa receptor.

U.S. Patent Nos. 4,638,047 and 4,772,686 to Szelke et al describe certain peptides (modified partial sequences of human fibrinogen) wherein an amide bond is replaced by a nonhydrolyzable isosteric linkage. These compounds are assertedly useful as thrombin inhibitors, and have the formula:

X Y Z A Pro Arg B W

wherein X and W could be hydrogen, Y could be D-Phe, Z could be L-Pro, A could be a keto dipeptide, and have a high binding activity for thrombin.

Summary of the Invention.

Surprisingly it has been found that by making certain modifications to the "B-W" portion to the prior art compounds, reversible, highly potent, highly selective factor IIa inhibitors useful in inhibiting the production of thrombin are produced.

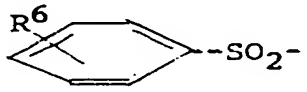
The invention thus includes compounds of the formula:

X Y Z A P Q T

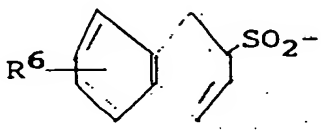
or a pharmaceutically acceptable salt thereof.

In this compound, X, when present, may be connected to T to form a ring compound, may be hydrogen, CH_3, an acyl group, or a general protective group (e.g. Boc, Z, or derivatives thereof).

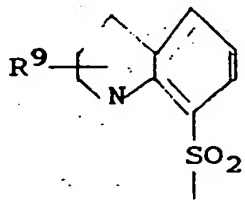
Y is D-Phe, D-diphenylalanyl, D-Val, D-Ile, or D-Nle, a phenylsulfonyl group of the formula:



a naphthylsulfonyl ("Dansyl") group of the formula:



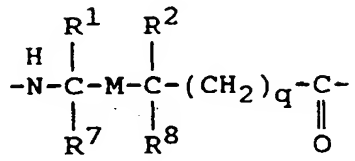
wherein R^6 is hydrogen, alkyl, N-alkyl or N-dialkyl, or a 8-(1,2,3,4-tetrahydroquinolinosulfonyl) compound of the formula:



wherein R^9 is hydrogen, or alkyl, especially lower (C_{1-6}) alkyl.

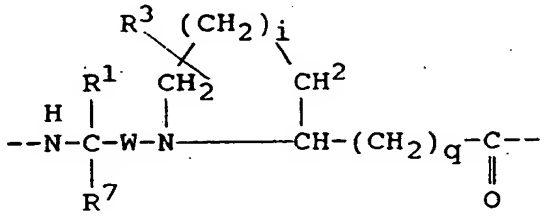
Z, when present, may be Gly, L/D-Ala, L/D-Val, L/D-Leu, Aib, L/D-Pro, or a substituted or unsubstituted L/D-Pro ring homologue.

A may be



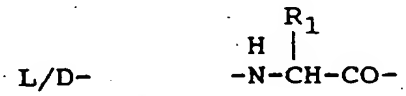
wherein M is $-(CO)_d-NH-$, $-(CO)_d-(CH_2)_p-$, $-(CO)_d-N(CH_3)--CH(OH)-(CH_2)_p-$,or $-CO-CF_2-(CO)_s-$, d is 0, 1 or 2, p is 0 to 5, q is 0 to 5, and s is 0 or 1. R^1 is an amino acid side chain that is characteristic of a hydrophobic, basic amino acid with an aliphatic or aromatic chain or spacer. R2 may be hydrogen, methyl, hydroxymethylene, or benzyl. R^7 and R^8 are independently selected from hydrogen, methyl, or lower (C_{1-3}) alkyl.

A may also be a proline-like group:



wherein W is $-CH_2-$, $-CO-(CH_2)_q-$, $-CH(OH)-(CH_2)_q-$, or $-CO-CF_2-(CO)_s$, i is 1 or 2, R^3 is hydrogen, CH_3, or COOH at ring CH_2 positions.

P and Q (if Q is present) are substituted or unsubstituted amino acids selected from the group consisting of L/D-Phe (e.g. including p-chlorophenylalanyl ("pclPhe"), homo-Phe ("HPhe") and L/D-Tyr), L/D-cyclohexylalaninyl, L/D-napthylalaninyl (1), L/D-napthylalaninyl (2), L/D-phenylglycinyl, L/D-Leu, L/D-Ile, L/D-Nle, L/D-Arg, L/D-Lys, L/D-His, homoarginine, homolysine, and pipecolic acid, D-diphenylalanyl, or



T may be connected to X, or may be -OH, $-OR^4$, $-NH_2$, $-NHR^4$, or $-NR^4R^5$, $-N(CH_2)_{1-6}NR^4R^5$, wherein R^4 and R^5 are independently selected from hydrogen, alkyl, aryl, (ar)alkyl, and wherein R^4 and R^5 can be cyclically bonded one to the other.

Due to their anti-factor IIa activity, these compounds have use in the manufacture of anticoagulant medicaments, especially ones intended for acute or initial administration. Once manufactured, the medicaments may be used in the treatment of mammals, including man. The medicaments are administered, on a regular basis, for example continually, to a mammal, believed to be suffering from a disease state susceptible to treatment by such medicaments. Such disease states include pulmonary embolism, thrombophlebitis, and arterial occlusion from thrombosis or embolism.

These compounds may also be used prophylactically to prevent further embolism, to forestall arterial and venous thrombosis, to prevent thromboemboli, and to prophylax against postoperative venous thrombosis or embolism.

The invention also includes a pharmaceutical composition further comprising a pharmaceutical carrier.

The invention further includes a process for preparing a compound of the formula, the process including coupling suitably protected amino acids or amino acid analogs, followed by removing the protecting groups.

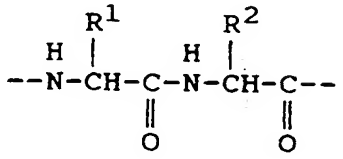
Description of the Preferred Embodiments

In various preferred embodiments of the invention, X is H, a general protective group (e.g. tertiary butyloxycarbonyl, or other protecting group).

Y is preferably D-phenylalanyl.

Z is preferably L- or D-prolinyl ("L/D-Pro").

A may be:

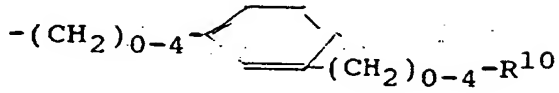


wherein R^1 goes to form L-Arg, but not D-Arg.

R^1 is

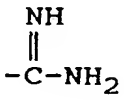
$-(CH_2)_{2-6}-R^{10}$

or

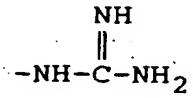


or

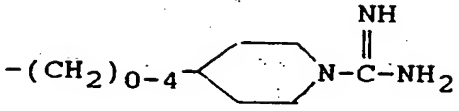
wherein R^{10} is $-NH_2$, or amidine:



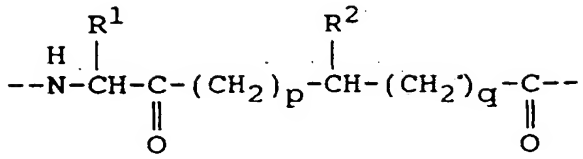
or guanadino:



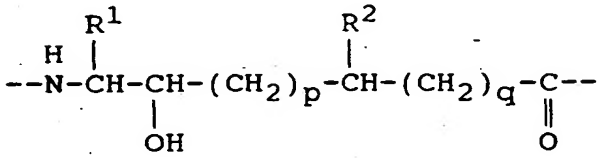
or R^1 is



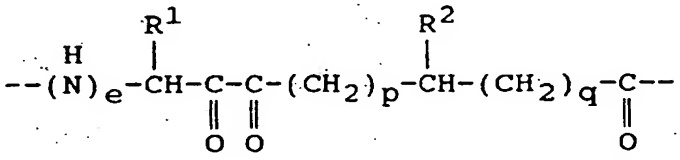
A is preferably the keto isostere:



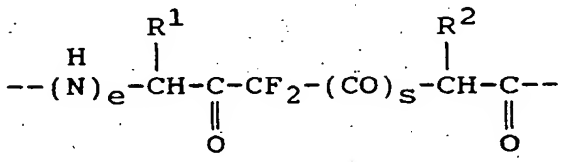
wherein p is preferably 1 and q is preferably 0. A may also be the hydroxy isostere:



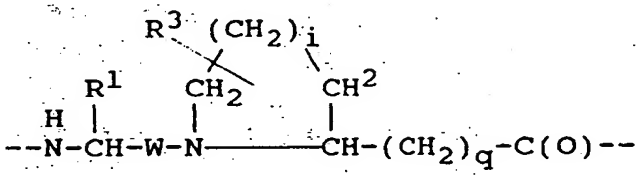
wherein again p is preferably 1, and q is preferably 0, or the diketo isostere:



or the keto-difluoro-methylene isostere:



wherein e is 0 or 1;
or A may be the proline-like group:



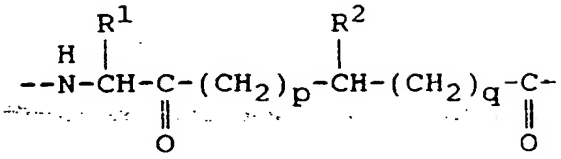
wherein R^1, R^2, R^3 and p, q, s and i have their previously given meanings. A is preferably however the keto isostere.

P may be as was previously defined, but preferably is L-phenylalaninyl or D-phenylalaninyl.

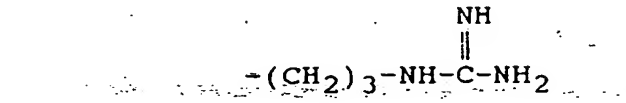
Q is preferably L/D-Lys or L/D-Arg.

T is preferably -OH, -OR^4 (wherein R^4 is aryl, alkyl, or aralkyl), or NR^4R^5 (wherein R^5 is alkyl, preferably methyl, ethyl or isopropyl).

In one of the most preferred embodiments of the invention, X is hydrogen, Y is D-Phe, Z is L-Pro, A is:



wherein R' is



As used herein the term "pharmaceutically acceptable salt" refers to salts that retain the desired biological activity of the parent compound and preferably do not impart any undesired toxic effects. Examples of such salts are acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid nitric acid, and the like. Salts may also be formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, and the like. Salts may be formed with polyvalent metal cations such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel and the like, or with an organic cation formed from N,N'-dibenzylethylenediamine or ethylenediamine, or combinations thereof (e.g. a zinc tannate salt).

Alkyl, as used herein, is preferably a saturated branched or unbranched hydrocarbon having one to six carbon atoms, e.g. methyl, ethyl, isopentyl, and allyl.

Aryl, as used herein, is an aromatic hydrocarbon group, preferably having 6 to 10 carbon atoms, such as phenyl or napthyl.

(Ar)alkyl, as used herein, is an arene group (having both aliphatic and aromatic portions), preferably having 7 to 13 carbon atoms, such as benzyl, ethylbenzyl, n-propylbenzyl, isobutylbenzyl.

A "substitution" with regard to the various amino acids (e.g. L/D-Phe, L/D-Cha, L/D-Nal(1), L/D-Nal(2), and L/D-phenylglycinyl) generally relate to substituting a group such as alkoxy, halogen, hydroxy, nitro, or lower alkyl onto an aromatic ring for a hydrogen that would usually be present. Substitutions can also be made on the alkyl chain connecting the aromatic portion to the peptide backbone, with, for instance lower alkyl groups substituting for a hydrogen. Still further substitutions can be made at the alpha position of an amino acid, also using an alkyl group.

Substitutions with regard to the amino acid phenylalanine include compounds such as L/D-homophenylalanyl, N methyl phenylalanyl, α-methyl-phenylalanyl, and α-methyl-tyrosyl.

Preferred substitutions involve the use of fluorine or chlorine as a halogen, and methoxy as an alkoxy group. With regard to alkyl and lower alkyl, generally alkyl groups having fewer (1 to 3) carbon atoms are preferred.

The compounds according to the general formula may be prepared in a manner conventional for such compounds. To that end, suitably N^{α} protected (and side-chain protected if reactive side-chains are present) amino acid derivatives or peptides are activated and coupled to suitably carboxyl protected amino acid or peptide derivatives either in solution or on a solid support. Protection of the α-amino functions generally takes place by urethane functions such as the acid-labile tert.-butyloxycarbonyl group (Boc), benzyloxycarbonyl(Z) group and substituted analogs or the base-labile 9-fluoremyl-methyloxycarbonyl (Fmoc). group. The Z group can also be removed by catalytic hydrogenation. Other suitable protecting groups include the Nps, Bmv, Bpoc, Aloc, MSC, etc. A good overview of amino protecting groups is given is given in The Peptides, Analysis, Synthesis, Biology, Vol. 3 E. Gross and J. Meienhofer, eds., (Academic Press, New York, 1981). Protection of carboxyl groups can take place by ester formation e.g. base-labile esters like methyl or ethyl, acid-labile esters like tert. butyl or, substituted, benzyl esters or hydrogenolytically. Protection of side-chain functions like those of lysine and glutamic or aspartic acid can take place using the aforementioned groups. Protection of thiol, and although not always required, of guandino, alcohol and imidazole groups can take place using a variety of reagents such as those described in The Peptides, Analysis, Synthesis, Biology id. or in Pure and Applied Chemistry, 59(3), 331-344 (1987). Activation of the carboxyl group of the suitably protected amino acids or peptides can take place by the

azide, mixed anhydride, active ester, or carbodizimide method especially with the addition of catalytic and racemization-suppressing compounds like 1-hydroxybenzotriazole, N-hydroxysuccinimide, 3-hydroxy-4-oxo-3,4-dihydro-1,2,3,-benzotriazine, N-hydroxy-5-norbornene-2,3-dicarboximide. Also the anhydrides of phosphorus based acids can be used. See, e.g. The Peptides, Analysis, Synthesis, Biology, supra and Pure and Applied Chem. 59(3), 331-344 (1987).

It is also possible to prepare the compounds by the solid phase method of Merrifield. Different solid supports and different strategies are known see, e.g. Barany and Merrifield in The Peptides, Analysis, Synthesis, Biology, Vol. 2, E. Gross and J. Meienhofer, eds., (Acad. Press, N.Y., 1980), Kneib-Cordonier and Mullen Int. J. Peptide Protein Res., 30, 705-739 (1987) and Fields and Noble Int. J. Peptide Protein Res., 35, 161-214 (1990). The synthesis of compounds in which a peptide bond is replaced by an isostere, can, in general, be performed using the previously described protecting groups and activation Procedures. Procedures to synthesize the modified isosteres are described in the literature e.g. for the $-CH_2-NH-$ isostere and for the $-CO-CH_2-$ isostere

Removal of the protecting groups, and, in the case of solid phase peptide synthesis, the cleavage from the solid support, can take place in different ways, depending on the nature of those protecting groups and the type of linker to the solid support. Usually deprotection takes place under acidic conditions and in the presence of scavengers. See, e.g. volumes 3, 5 and 9 of the series on The Peptides Analysis, Synthesis, Biology, supra.

Another possibility is the application of enzymes in synthesis of such compounds; for reviews see e.g. H.D. Jakubke in The Peptides, Analysis, Synthesis Biology, Vol. 9, S. Udenfriend and J. Meienhofer, eds., (Acad. Press, N.Y., 1987).

However made, the compounds are useful for the manufacture of medicaments which have use in treating disease states involving undesired blood coagulation. In such a case the particular compound synthesized will typically be associated with a pharmaceutical carrier. Pharmaceutical carriers vary from things as relatively simple as sterilized water for injection to things as relatively complicated as microspheres and biodegradable implants.

As medicaments, the compounds are preferably administered subcutaneously, topically, intranasally, intravenously, intramuscularly or locally (e.g. via an implant). Depot administration is also possible. However certain of the compounds (e.g that described in EXAMPLE VII.d.) may be administered via an oral dosage form.

The exact dose and regimen for administration of these compounds and compositions will necessarily be dependent upon the needs of the individual subject to whom the medicament is being administered, the degree of affliction or need, and of course, the judgment of the medical practitioner. In general parenteral administration requires lower dosages than other methods of administration which are more dependent upon absorption. Illustratively however, the dosages are in the range of 0.01 to 10 mg per kilogram body mass.

The medicament manufactured with the compounds may also be used as adjuvant in acute anticoagulant therapy. In such a case, the medicament is administered with other compounds useful in treating such disease states.

The compounds may also be used with implantable pharmaceutical devices such as those described in US Patent 4,767,628, the contents of which are incorporated by this reference. Then the device will contain sufficient amounts of compound to slowly release the compound (e.g. for more than a month).

Methods of making medicaments which can be adapted to contain the compound for parenteral administration are described in the standard reference, Chase et al., Remington's Pharmaceutical Sciences, (16th ed., Mack Publishing Co., Easton. PA, U.S.A., 1980) at pages 1463 through 1497.

The invention is further explained by reference to the following illustrative EXAMPLES.

EXAMPLES

If no configuration of the amino acid has been stated, the L form is intended.

I. The following abbreviations have been used for the various groups employed:

tBu	= tertiary butyl
Me	= methyl
Z	= benzyloxycarbonyl

II. The following abbreviations have been assigned to the solvents or reagents used:

THF	= tetrahydrofuran
DCM	= dichloromethane
MeOH	= methanol

EA = ethylacetate
OtBu = tertiary butoxy
Mtr = 4-methoxy-1,2,5-trimethylbenzenesulfonyl
Tol = toluene
EtOH = ethanol
Bu = butanol
HOAc = acetic acid
DMF = N,N-dimethylformamide
DCC = dicyclohexylcarbodi-imide
DCU = dicyclohexylurea
TFA = trifluoro-acetic acid
N.E.M. = N-ethylmorpholine
HOBt = 1-hydroxybenztriazole

III. The following abbreviations have been used throughout this specification for the amino-acid groups:

Phe = phenylalanyl
Pro = prolyl
Arg = argininyl
Asp = aspartyl
Glu = glutamyl
Gly = glycinyl
His = histidinyl
Lys = lysinyl
Tyr = tyrosinyl
Ile = isoleucinyl
Nle = norleucinyl
Cha = cyclohexylalanyl
Val = valyl
Leu = leucyl
Ala = alanyl
Aib = aminoisobutyric acid
D-DPA = diphenylalanyl
Har = homoarginine
Hly = homolysine
Pec = pipecolic acid
Nal(1) = napthylalaninyl (1)
Nal(2) = napthylalaninyl (2)
pClPhe = p-chlorophenylalanyl
HPhe = homo-phenylalanyl

IV. All sequences mentioned herein are written according to the generally accepted convention wherein the N-terminal amino acid is on the left and the C-terminal amino acid is on the right.

EXAMPLE I

1.1 Z-D-Phe-Pro-Arg-Gly-Phe-OMe

A solution of the partially protected peptide Z-D-Phe-Pro-Arg-OH 0.5 g (0.9 mmol) in 5 ml of DMF is cooled to 0 °C and 220 mg (1.6 mmol) of 1-hydroxybenzotriazole and 185 mg (0.9 mmol) of dicyclohexylcarbodiimide are added successively with stirring. The reaction mixture is stirred for 30 minutes; then a solution of H-Gly-Phe-OMe·HCl in 5 ml of DMF with sufficient triethylamime to give a pH of 7 is added. The mixture is stirred for 16 hours at room temperature. Thereafter, the dicyclohexylurea formed is removed by filtration. After evaporation of the filtrate, the residue is dissolved in water and extracted with CH_2Cl_2, the crude product is purified on SiO_2 with EA/pyridine/HOAc/H2O 6:1.5:1.5:1 (by vol.).

1.2 Z-D-Phe-Pro-Arg-Gly-Phe-OH

The methylester of 1.1 (0.2 g = 0.25 mmol) is removed by treatment in dioxane and water at room temperature with sufficient 1N NaOH solution to give a pH of 13 for 1 hr. After acidification, the mixture is evaporated and extracted with methylene chloride to give 180 mg of the corresponding free acid.

1.3 H-D-Phe-Pro-Arg-Gly-Phe-OH

Hydrogenolysis of the partially protected pentapeptide (180 mg = 0.24 mmol) using 18 mg of Pd/C 10% as the catalyst in methanol with 2 equivalents of 1N HCl gives the free title compound.

The material thus obtained was treated with an ion-exchange resin in the acetate form (Dowex) to convert the pentapeptide into the acetate salt. After removing the resin by filtration, the filtrate was lyophilized and the product purified by chromatography on SiO_2 with an EA/pyridine/HOAc/H_2O (6:2:2:1, by vol.) eluent. The pooled fractions were evaporated and lyophilized to give 80 mg of analytically pure product. Spectral data was in agreement with the assigned structure. Rf in EA/pyridine/HOAc/H_2O 6:2:2:1 = 0.44 (on SiO_2).

EXAMPLE II

2.1 Z-Arg-Ala-OMe

The synthesis of 2.1 was carried out according to the method of Kraniova Bt. et al (Zh Obskch Khim, 39, 92 (1969).

2.2 Z-Arg-Ala-Phe-OtBu

Z-Arg-Ala-Ome was saponified using the prescription for compound 1.3. The free acid was coupled with H-Phe-OtBu using the same procedure described in EXAMPLE 1.2.

2.3 Boc-D-Phe-Pro-Arg-Ala-Phe-OtBu

A solution of 0.72 g (1.99 mmol) Boc-D-Phe-Pro•OH in 16 ml of DMF is cooled to 0 °C and 540 mg (3.99 mmol) of 1-hydroxy benzotriazole and 460 mg (2.2 mmol) of dicyclohexylcarbodiimide are added. The reaction mixture is stirred for 30 minutes. Then a solution of H-Arg-Ala-Phe-OtBu (obtained by catalytic hydrogenolysis of Z-Arg-Ala-Phe-OtBu in 16 ml of DMF with 2 eq. of 1N HCl) in 16 ml of DMF with sufficient N.E.M. to give a pH of 7 is added. The mixture is stirred for 16 hours at room temperature. The dicyclohexylurea formed is filtered off and the filtrate concentrated by evaporation. The residue is dissolved in CH_2Cl_2 and washed with 5% $NaHCO_3$ solution, 5% $KHSO_4$ solution and water. The organic phase is dried over anhydrous Na_2SO_4 and evaporated.

2.4 H-D-Phe-Pro-Arg-Ala-Phe-OH

The protecting groups of the pentapeptide of 2.3 are removed by treatment with 15 ml of 90% TFA in the presence of 0.6 ml of anisole. The mixture is stirred for 1 hour at room temperature. The material thus obtained is dissolved in t-BuOH/water (1:1, v/v) and treated with an ion-exchange resin in the acetate form. After filtering the resin, the filtrate is lyophilized and the product purified by chromatography on silica with a Bu/pyridine/ HOAc/H_2O (16:3:4:1, by vol.) eluent. The fractions containing the desired material are evaporated and then lyophilized to give 510 mg of the titled compound. Spectral data was in agreement with the assigned structure. Rf in Bu/pyridine/HOAc/H_2O 8:3:1:4 = 0.45 (on SiO_2).

2.5 Boc-D-Phe-Pro-Arg-Ala-Phe-Lys(Boc)OtBu.

Using the prescription for compound 2.3, 0.8 mmol of Boc-D-Phe-Pro-OH was coupled with 0.65 g (0.8 mmol) of Z-Arg-Ala-Phe-Lys(Boc)-OtBu.

2.6 H-D-Phe-Pro-Arg-Ala-Phe-Lys-OH.

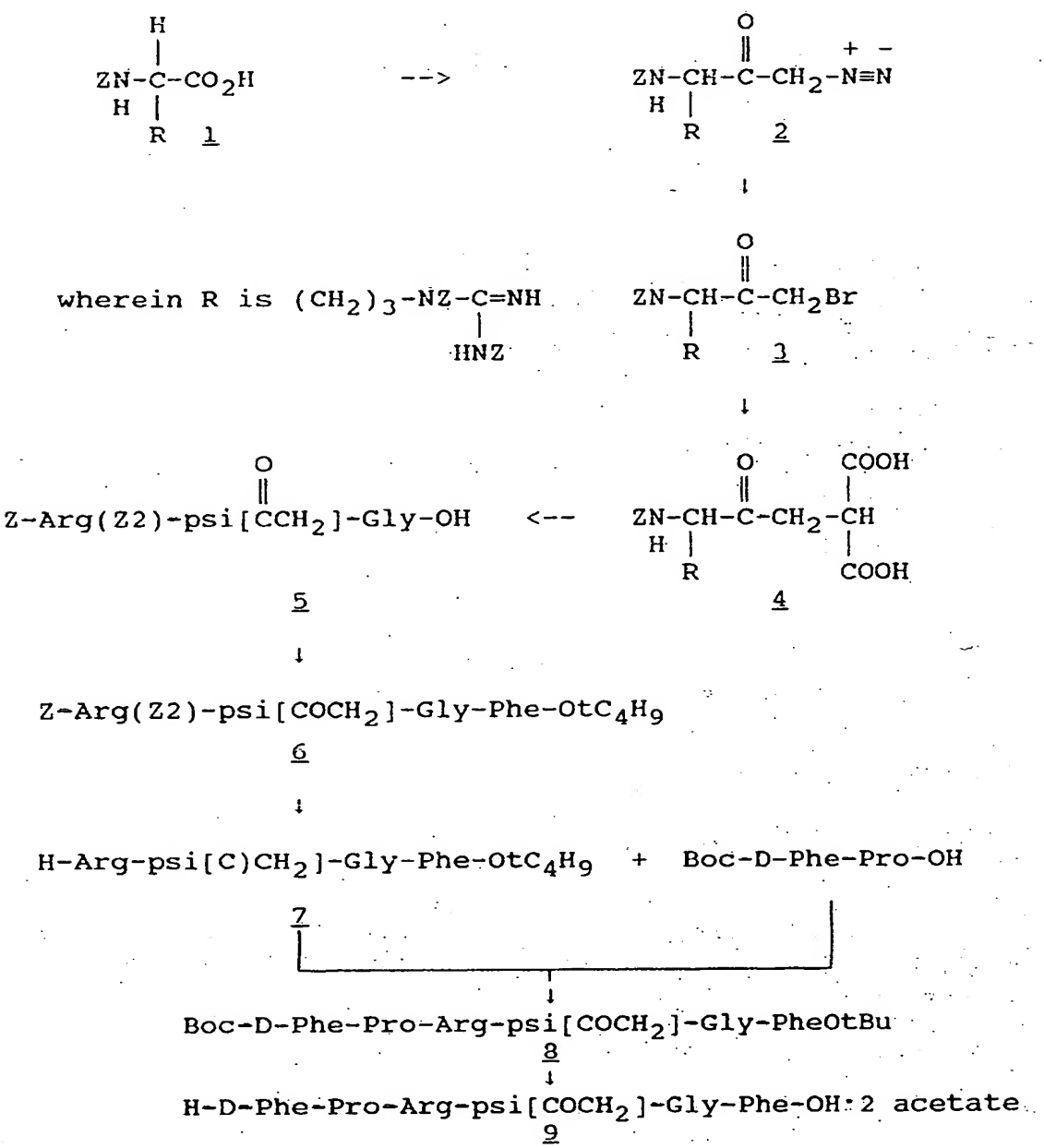
The protecting groups of the pentapeptide 2.5 are removed using the prescription for compound 2.4. The analytically pure compound (0.1 g) was obtained, the spectral data of which agreed with the assigned structure. Rf in Bu/pyridine/HOAc/H_2O (8:3:1:4) = 0.3 (on SiO_2).

EXAMPLE III

H-D-Phe-Pro-Arg-Gly-Phe-Lys-OH was prepared using a prescription analogous to that for compound

2.6. Analytical results agreed with the assigned structure. Rf in Bu/pyridine/HOAc/H_2O 4:1:1:2 = 0.4 (on SiO_2).

EXAMPLE IV



4.1 Z-Arg(Z_2)-CH_2Br

Nα, Nδ, NΩ-tri-benzyloxycarbonyl-L-Arg was obtained from Nα-benzyloxycarbonyl-L-Arg by the method of Wünsch and Wendlberger (Chem. Ber., 100, p. 160 (1967)) in 30 % yield. Rf on SiO_2 is 0.43 in toluene/EtOH (8:2).

Compound 1 (36.0 mmol) was dissolved in dry THF and the mixed anhydride was prepared with isobutylchloroformate and N.E.M. An ethereal solution of diazomethane was added in several portions, and after stirring for 20 hours, the diazomethylketone 2 was formed. Rf is 0.67 (Tol/EtOH, 8:2) on SiO_2.

The reaction mixture was cooled to 0° C and an ethereal solution of HBr was added and the reaction

was followed by TLC. After extraction with water and ether, compound 3 could be obtained in crystal form. Rf on SiO_2 is 0.81 (Tol/EtOH, 8:2).

4.2 Z-Arg (Z_2)-psi[CO-CH_2]Gly-OH (5)

To a solution of compound 3 in dry THF (17.54 mmol in 75 ml) a solution of the sodium salt of di(t-butyldimethylsilyl)malonate in THF was added dropwise with stirring. The coupling was complete after 3 hours at room temperature. In situ acidic removal of the ester groups followed by extraction with CH_2Cl_2 and subsequent evaporation gave compound 4 as a yellow oil. Rf on SiO_2 is 0.51 (CH_2Cl_2/MeOH, 8/2).

Decarboxylation of compound 4 was carried out by refluxing in toluene for 1 hour. After chromatography, the pure ketomethylene isosteric dipeptide 5 was obtained as a white amorphous powder. Rf on SiO_2 is 0.17 (CH_2Cl_2/MeOH, 9/1).

4.3 H-Arg-psi[CO-CH_2]-Gly-PheOtBu (7)

HOBt was added (0.58 mmol, 79.1 mg) to a solution of 5 (0.39 mmol, 0.25 g) in DMF (1.0 ml). The solution was cooled to 0 °C and DCC (0.43 mmol, 88.7 mg) was added. After 1 hour of stirring, a solution of H-Phe-OtBu•HCl (0.59 mmol, 0.21 g) in DMF, adjusted to pH 7.5 with N.E.M., was added. The mixture was then stirred until no starting compound 5 was detectable via TLC. The reaction mixture was cooled to -20 °C and the precipitated DCU filtered off. The filtrate was concentrated and the residue dissolved in CH_2Cl_2. The organic layer was washed with Na_2CO_3 and $KHSO_4$ solution. After washing with water, the organic layer was dried over Na_2SO_4. Filtration and evaporation yielded 6 in 92%. Rf(6) = 0.60 (Tol/EtOH 8/2).

Crude product 6 (119 mg, 0.14 mmol) was dissolved in DMF, and after adding 50 mg Pd/C (10%), H2 was bubbled through the mixture until no starting compound was left. Two equivalents of HCl were added (0.28 ml, 2 N HCl). Compound 7 was obtained quantitatively, after filtering off the catalyst and concentrating the filtrate. Rf(7) is 0.33 in EA/pyridine/HOAc/H_2O (6/2/2/1).

4.4 H-D-Phe-Pro-Arg-psi[$COCH_2$]-Gly-Phe-OH (9)

Boc-D-Phe-Pro-OH was dissolved in DMF (0.20 mmol, 72.7 mg) and coupled to 7 (0.14 mmol, 89.6 mg) via a HOBt (0.3 mmol, 40 mg)/DCC (0.22 mmol, 41.4 mg) mediated coupling as described for compound 6. Compound 8 was obtained. Rf = 0.27 in EA/pyridine/HOAc/H_2O (80/20/6/5).

To the crude product 8 (135 mg), 90% TFA was added (5 ml) and anisole as a scavenger. After 1 hour, the reaction was complete, and the mixture was poured into ether. The resulting precipitate was filtered off, washed with ether, and dissolved in water/t-butanol (1/1). Dowex Ac^- was added to exchange the TFA anion with acetate anions. The ion exchanger was removed by filtration and the product 9 was freeze-dried. This product was purified by column chromatography Bu/pyridine/HOAc/H_2O (4/0.75/0.25/1). Rf(9) = 0.19.

EXAMPLES V & VI

H-D-Phe-Pro-Arg-Gly-His-OH and H-D-Phe-Pro-Arg-psi[$COCH_2$]-Gly-Phe-Lys-OH were prepared in manners similar to EXAMPLE I and EXAMPLE IV respectively.

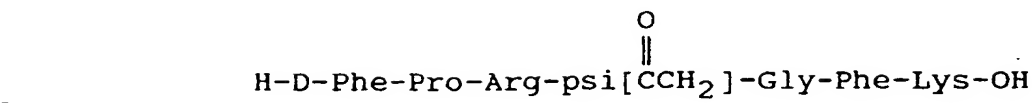
EXAMPLE VII

In a similar manner the following diketo isosteres are prepared:

a) H-D-Phe-Pro-Arg-CO-Phe-OH;
b) H-D-Phe-Pro-Arg-CO-Phe-Lys-OH;
c) H-D-Phe-Pro-Arg-CO-Gly-Phe-Lys-OH; and
d) H-D-Phe-Pro-Arg-CO-D-Phe-D-Tyr-OH.

EXAMPLE VIII

Synthesis of

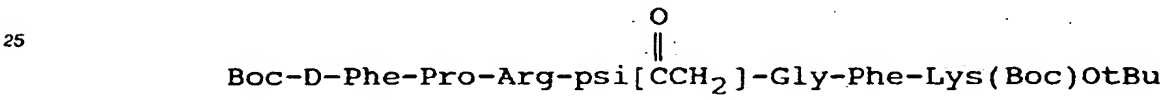


H-D-Phe-Pro-Arg-psi[CCH$_2$]-Gly-Phe-Lys-OH

a. Compound 5 of EXAMPLE 4.2 (1.00 g, 1.58 mmol) was activated with HOBt (0.32 g, 2.39 mmol) and DCC (0.36 g, 1.74 mmol) in DMF (10 ml @ 0°C). A solution of H-Phe-Lys(Boc)OtBu (2.49 mmol) in DMF (10 ml) was adjusted to pH 7.5 with NEM and added to the activated compound 5, which had been stirred for 1 hour at room temperature. The mixture was stirred until no compound 5 could be detected on TLC. The reaction mixture was worked up in the same manner as compound 6 in EXAMPLE 4.3, to obtain compound 10 (i.e. Z-Arg(Z)$_2$-psi[CO-CH$_2$]-Gly-Phe-Lys(Boc)OtBu) in 85%. Rf(compound 10) = 0.29 (DCM/EA. 8/2).

b. The crude product 10 was dissolved in DMF (0.47 mmol, 0.5 g in 10 ml). After adding Pd/C (50 mg) H_2 was bubbled through the solution until no starting compound was detectable on TLC. Two equivalents of HCl were added before removing the catalyst by filtration. Compound 11 (i.e. H-Arg psi[CO-CH$_2$]-Gly-Phe-Lys(Boc)OtBu) was kept in solution and immediately used in the synthesis of compound 12. Rf-(compound 11) = 0.43 (EA/Pyridine/HOAc/water, 6/2/2/1).

c. Boc-D-Phe-Pro-OH was dissolved in DMF (0.705 mmol, 256 mg) cooled to 0°C and HOBt (1.05 mmol, 143 mg) and DCC (0.78 mmol, 160 mg) were added. The same procedure was followed as described in this EXAMPLE VIII.a., above, to give compound 12, i.e.:



Boc-D-Phe-Pro-Arg-psi[CCH$_2$]-Gly-Phe-Lys(Boc)OtBu

Rf(compound 12) = 0.27 in (EA / pyridine / HOAc / water 6/2/2/1).

d. Compound 12 was then dissolved in 90% TFA (2.5 ml) and anisole was added as a scavenger. After 1 hour, the mixture was worked up as described in EXAMPLE 4.4 to give compound 13, i.e. the titled compound (H-D-Phe-Pro-Arg-psi[COCH$_2$]-Gly-Phe-Lys-OH), finally as an acetate salt. Compound 13 was freeze-dried and purified by column chromatography. Rf(compound 13) = 0.12 (EA/pyridine/HOAc/water 6/2/2/1). Compound 13 is a highly potent, highly selective Factor IIa inhibitor.

EXAMPLE IX

The following compounds were prepared, and all had Factor IIa binding activity:

a. H-D-Nle-Pro-Arg-Ala-Phe-Lys-OH;
b. H-D-Phe-Val-Arg-Ala-Phe-Lys-OH;
c. H-D-Phe-Pro-Arg-Ser-Phe-Lys-OH;
d. H-D-Phe-Pro-Arg-Ala-Cha-Lys-OH;
e. H-D-Phe-Pro-Arg-Ala-Nal(1)-Lys-OH;
f. H-D-Phe-Pro-Arg-Ala-Nal(2)-Lys-OH;
g. H-D-Phe-Pro-Arg-Ala-pClPhe-Lys-OH;
h. H-D-Phe-Pro-Arg-Ala-D-αMeTyr-Lys-OH;
i. H-D-Phe-Pro-Arg-Ala-L-αMeTyr-Lys-OH;
j. H-D-Phe-Pro-Arg-Ala-HPhe-Lys-OH;
k. H-D-Phe-Pro-Arg-Ala-Phe-Pec-OH; and
l. H-D-Phe-Pro-Arg-Ala-Phe-Arg-OH.

EXAMPLE X

The following compounds are prepared in a similar manner:

a. H-D-Phe-Pro-Arg-Ala-aMePhe-Lys-OH,
b. H-D-Ile-Val-p-AmPhe-Ala-Cha-Arg-OH, and
c. H-D-Phe-Pro-Arg-psi[COCH$_2$]-Ala-Nal(2)-Lys-OH.

EXAMPLE XI

FACTOR IIa INHIBITORY ACTIVITY

The inhibition of human thrombin was investigated by continuously monitoring the splitting of the chromogenic substrate S 2238 (N-D-Phe-L-pipecolyl-L-Arg-p-nitro-anilide 2 HCl) in the absence and in the presence of 3, 1, 0.3, 0.1 and 0.03 mM of the compound investigated. These measurements were performed with the help of a kinetic microtiter plate reader. From these measurements the end absorbances were calculated after 90 minutes. Based on these total scores, an IC_{50} of the various compounds investigated is expressed as the molar concentration which inhibited the end absorbance by 50%. IC50 values for aXa-activity were investigated in the same way by using the chromogenic substrate s2222 (N-benzoyl-Ile-Glu-(OCH_3)Gly-Arg-pNa).

IC 50 values measured after 90 minutes and the ratio alla over aXa.

COMPOUND	IC50 alla	IC50 aXa	IC50 alla:aXa
H-D-Phe-Pro-Arg-Gly-OH	4.0E-3	4.8E-3	1
H-D-Phe-Pro-Arg-Gly-Phe-OH	4.1E-4	1.3E-3	3
H-D-Phe-Pro-Arg-Gly-Phe-Lys-OH	2.5E-4	1.6E-3	6
H-D-Phe-Pro-Arg-Ala-Phe-Lys-OH	1.1E-4	3.1E-3	28
H-D-Phe-Pro-Arg-psi[$COCH_2$]-Gly-Phe-OH	3.7E-5	2.1E-3	57

EXAMPLE XII

The following data shows minimum inhibitory concentrations (K_i[M]) of various compounds of the invention in comparison with a prior art compound (EXAMPLE XII.a), and the effect of incorporating an isosteric linkage into the peptides.

COMPOUND	K_i[M]-Factor IIa
a. H-D-Phe-Pro-Arg-Gly-OH	7.0×10^{-5}
b. H-D-Phe-Pro-Arg-psi[$COCH_2$]-Gly-OH	2.6×10^{-5}
c. H-D-Phe-Pro-Arg-Gly-Phe-OH	7.6×10^{-6}
d. H-D-Phe-Pro-Arg-psi[$COCH_2$]-Gly-Phe-OH	4.8×10^{-7}
e. H-D-Phe-Pro-Arg-Gly-Phe-Lys-OH	3.4×10^{-6}
f. H-D-Phe-Pro-Arg-psi[$COCH_2$]-Gly-Phe-Lys-OH	2.8×10^{-8}

References herein to specific Examples or embodiments should not be interpreted as limitations to the invention's scope which is determined by the claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Akzo, nv
(B) STREET: Velperweg 76
(C) CITY: Arnhem
(E) COUNTRY: Netherlands
(F) POSTAL CODE: NL 6824 BM

(ii) TITLE OF INVENTION: Factor IIa Inhibitors.

(iii) NUMBER OF SEQUENCES: 3

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide derivative

(ix) FEATURES
(D) OTHER INFORMATION:
bonded to A1 is a dansyl, phenylsulfonyl, or 8-(1,2,3,4-tetrahydroquinolinosulfonyl) group;
A1 may be Arg, Xaa, or Lys;
A2 may be Gly, Ala, Phe, or Ser;
P may be Phe, Xaa, Leu, Ile, Arg, Lys, or His;
Q may be Phe, Xaa, Leu, Ile, Arg, Lys, His.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

A1 A2 P Q
1

(3) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide derivative

(ix) FEATURES
(D) OTHER INFORMATION:
bonded to Z is a dansyl, phenylsulfonyl, or 8-(1,2,3,4-tetrahydroquinolinosulfonyl) group;
Z may be Xaa, Gly, Ala, Val, Leu, or Pro;
A1 may be Arg, Xaa, or Lys;
A2 may be Gly, Ala, Phe, or Ser;
P may be Phe, Xaa, Leu, Ile, Arg, Lys, or His.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Z A1 A2 P
1

(4) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide derivative

(ix) FEATURES
(D) OTHER INFORMATION:
bonded to Z is a dansyl, phenylsulfonyl, or 8-(1,2,3,4-tetrahydroquinolinosulfonyl) group;
Z may be Xaa, Gly, Ala, Val, Leu, or Pro;
A1 may be Arg, Xaa, or Lys;
A2 may be Gly, Ala, Phe, or Ser;
P may be Phe, Xaa, Leu, Ile, Arg, Lys, or His;
Q may be Phe, Xaa, Leu, Ile, Arg, Lys, or His.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Z A1 A2 P Q
1 5

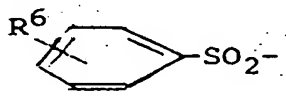
Claims

1. A compound of the formula:

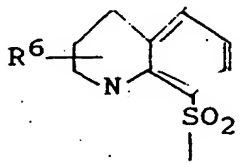
X Y Z A P Q T

or a pharmaceutically acceptable salt thereof; wherein X, when present, is H, CH_3, an acyl group, a general protective group, or connected to T;

Y is a D-amino acid selected from the group consisting of D-Phe, D-DPA, D-Val, D-Ile, and D-Nle, or Y is Dansyl, a group of the formula:



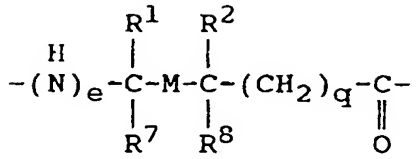
or a group of the formula:



wherein R^6 is hydrogen, alkyl, N-alkyl or N-dialkyl;

Z, when present, is Gly, a substituted or unsubstituted L/D-Pro ring homologue, L/D-Ala, L/D-Val, L/D-Leu, Aib, or L/D-Pro;

A is



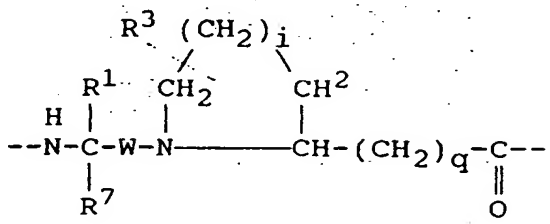
wherein

M is -(CO)$_d$-NH-, -(CO)$_d$-(CH$_2$)$_p$-, -(CO)-N(CH$_3$)-, -CH(OH)-(CH$_2$)$_p$-, or -CO-CF$_2$-(CO)$_s$-, d is 0, 1 or 2, p is 0 to 5, q is 0 to 5, e is 0 or 1, and s is 0 or 1,

R^1 is an amino acid side chain that is characteristic of a hydrophobic, basic amino acid with an aliphatic or aromatic chain,

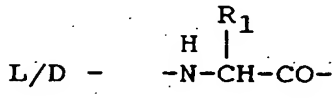
R^2 is hydrogen, methyl, hydroxymethylene, or benzyl,

R^7 and R^8 are independently selected from the group H, CH$_3$, and lower (C$_{1-3}$) alkyl, or A is



wherein W is -CH$_2$-, -CO-(CH$_2$)$_q$-, -CH(OH)-(CH$_2$)$_q$-, or - CO-CF$_2$-(CO)$_s$-, i is 1 or 2, R^3 is H, CH$_3$, or COOH at ring CH$_2$ positions;

Q, when present, and P are substituted or unsubstituted amino acids, said amino acids independently selected from the group consisting of L/D-Phe, L/D-Cha, L/D-Nal(1), L/D-Hal(2), L/D-phenylglycinyl, L/D-Leu, L/D-Ile, L/D-Nle, L/D-Arg, L/D-Lys, L/D-His, Har, Hly, and Pec, D-DPA, or Q and P or either of them may be



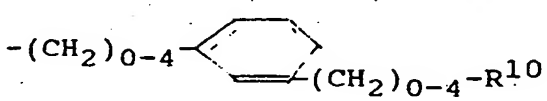
and

T is -OH, -OR^4, -NH^2, -NHR^4, -N(CH$_2$)$_{1-6}$NR^{4}R^5, or -NR^{4}R^5, wherein R^4 and R^5 are independently selected from hydrogen, alkyl, aryl, (ar)alkyl, and wherein R^4 and R^5 can be cyclically bonded one to the other, or T is connected to X.

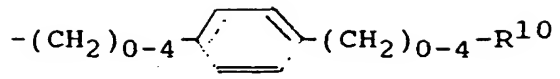
2. The compound of claim 1 wherein X is hydrogen, and R^1 is

-(CH$_2$)$_{2-6}$-R^{10}

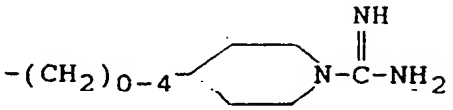
or



or



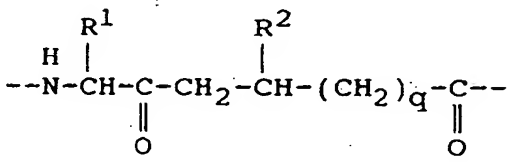
wherein R^{10} is $-NH_2$, or amidine, or guanadine,
or R^1 is



3. The compound of claim 1 wherein Y is D-Phe.

4. The compound of claim 1 wherein Z is L-Pro or D-Pro.

5. The compound of claim 1 wherein A is the ketomethylene isostere:



6. The compound of claim 1 wherein P is L-Phe.

7. The compound of claim 1 wherein Q is L-Lys or L-Arg.

8. A compound having selective Factor IIa binding activity of the formula:

D' A^1 A^2 Arg E A^3 A^4 A^5 F

wherein D' is Boc, benzyloxycarbonyl, or hydrogen;

- A^1 is D-Phe or D-Nle;
- A^2 is Pro or Val;
- A^3 is Gly, Ala, Nal(1), Nal(2), p-ClPhe, L/D-αMeTyr, HPhe, Phe, or Ser;
- E is either an amide bond or an isosteric linkage between Arg and A^3;
- A^4 is Arg, Lys, Phe, Pec, His, or Cha;
- A^5 is Arg, Lys, or Pec; and
- F is -OH, $-OR^4$, $-NH^2$, $-NHR^4$, $-N(CH_2)_{1-6}NR^4R^5$, or $-NR^4R^5$, wherein R^4 and R^5 are independently selected from H, alkyl, aryl, (ar)alkyl, or wherein R^4 and R^5 are cyclically bonded to one another.

9. A pharmaceutical composition comprising a pharmaceutical carrier and the compound of claim 1 or claim 8.

10. A process for preparing a compound of claim 1, said process comprising: coupling suitably protected amino acids or amino acid analogs, followed by removal of the protecting groups.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 92 20 0312

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
D,X	EP-A-0 118 280 (M. SZELKE C.S.) 12 September 1984 * the whole document * ---	1-10	C07K5/02 C07K7/02 C07K5/08
X	BIOCHEMISTRY. vol. 29, no. 30, 31 July 1990, EASTON, PA US pages 7095 - 7101; J.M.MARAGANORE C.S.: 'DESIGN AND CHARACTERIZATION OF HIRULOGS:A NOVEL CLASS OF BIVALENT PEPTIDE INHIBITORS OF THROMBIN' * the whole document * ---	1-10	C07K7/06 C07K7/64 A61K37/64
X	HAEMOSTASIS vol. 19, no. 2, 1989, BASEL pages 74 - 82; K.KRUPINSKI C.S.: 'ANTITHROMBOTIC EFFECTS OF THREE INHIBITORS IN A RAT MODEL OF LASER-INDUCED THROMBOSIS' The whole document; espec. pag.76 and tab.2 ---	1,9,10	
P,X	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. vol. 177, no. 3, 28 June 1991, DULUTH, MINNESOTA US pages 1049 - 1055; T.KLINE C.S.: 'HIRULOG PEPTIDES WITH SCISSILE BOND REPLACEMENTS RESISTANT TO THROMBIN CLEAVAGE' * the whole document * -----	1-10	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

C07K
A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	10 APRIL 1992	GROENENDIJK M.S.M.

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